Feasibility of Enzymatic Assay for ATP as an Indicator of Subterranean Microbial Life on Mars.

Andrew W. Szumlas, Francisco Andrade, Lisa M. Pratt, and Gary M. Hieftje
Indiana University, Departments of Chemistry and Geology, Bloomington, IN 47405
aszumlas@indiana.edu

With the stunning success of the recent Mars Rover missions, interest in the detection of life on the Red Planet has been renewed. Specifically, evidence in the geology of the Martian surface seems to indicate that water was once abundantly available. It has been postulated that although the surface of Mars is currently cold and barren, below the surface of the planet lie subterranean water sources. It is this water table that presents the most likely place for microbial life on Mars. This poster will discuss a method to reach this underground water, and will focus on the use of an enzymatic assay for the detection of the biochemical marker adenosine triphosphate (ATP).

In the search for living organisms on other planets, it is useful to consider common traits of life as we know it. The ubiquitous energy molecule ATP provides an excellent target and indicator of cellular life forms. A sensitive assay exists for ATP that takes advantage of a natural enzyme, firefly luciferase. This enzyme, when combined with the substrate luciferin and two other cofactors, catalyzes a reaction that efficiently releases light (quantum efficiency >90%). The use of this enzyme represents a simple, selective, and sensitive means to determine the presence of ATP, and in turn, living organisms. However, the implementation of this assay on or below the surface of Mars is not as straightforward as performing the assay in a laboratory. As was stated previously, any microorganisms are likely to be concentrated in subterranean reservoirs of liquid or frozen water. Thus, to effectively test for these microorganisms with the luciferase assay, one must first drill through frozen layers. Also, since this water has slowly receded through rock layers, it is likely to be highly saline, with high contents of sodium, calcium, and other inorganic ions. This brine solution presents a challenge in that the enzymatic nature of the ATP assay can be adversely affected by the salinity of the surrounding liquid. Steps must then be taken to mitigate the effects of the brine on the assay.

The focus of this poster will be on the development of a method to employ the firefly luciferase ATP assay for the detection of microorganisms on Mars. Strategies to overcome interference effects of the concentrated brine solution will be discussed, along with preliminary results in this area. Our strategies include two main methods, the first of which involves removing bacteria from the brine solution, followed by bacterial lysis in a buffer compatible with the luciferase enzyme. The second strategy attempts to simplify the analysis by working directly in the brine medium. To protect the enzyme, micellar surfactants and other chemical enhancers can be added in order to shield the enzyme from the negative effects of the brine components. An overview of these strategies along with conceptual drawings of how the equipment can be integrated into a compact module will be provided.